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Occurrence of *Paragonimus harinasutai* in Zhejiang, China

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Abstract

Large metacercariae were found in the crab, *Sinopotamon chekiangenes*, in Ninghai County, Zhejiang, China, in 1985. The metacercariae were pinkish in color with two cyst walls, and $525.2 \pm 38.6 \times 500.2 \pm 31.7 \mu\text{m}$ in size. Adult worms recovered experimentally from the lungs of cats were primary-identified as *Paragonimus harinasutai*. In 2004, specimens were sent to Mahidol University, Thailand, for re-identification, and it was confirmed that the species was *P. harinasutai* Miyazaki & Vajrasthira, 1968. The ratio of width to length for the adult worm is $1 : 2.8 \pm 0.2$. The ventral sucker is larger than the oral, at a ratio of $1.3 \pm 0.1 : 1$. The ovary and testes have 4-6, and 4-5, main branches, respectively; each branch is further divided into more delicate branches. The spines are mixed, single-spaced anteriorly, with double spines between the ventral sucker and testes. Small variations in morphology were found between the specimens from China and Thailand. The specimens are kept in the Zhejiang Academy of Medical Sciences, China.

Keywords: *Paragonimus harinasutai*, description, experimental infection, China

Introduction

Paragonimus is an important human trematode in some areas of the world, especially in the Far East. There are more than 40 species of *Paragonimus*, which have been found in Korea, Japan, China, Thailand, the Philippines, Indonesia, Cameroons, Congo, Liberia, Nigeria, India, some Pacific islands, Peru and Ecuador [1-2]. In China, the prevalence of infection ranges from 15-45% in endemic fields [3]. *P. westermani* is a common species in Zhejiang, and *P. westermani*, *P. skrjabini* and

P. heterotremus are common in China; all could cause human paragonimiasis. All of the *Paragonimus* metacercariae found in China are different from *P. harinasutai*. In Zhejiang, four species of lung flukes have been reported, *P. westermani*, *P. skrjabini*, *P. harinasutai* and *Euparagonimus cenocopiosis* [4]. In this paper, we report *P. harinasutai* from experimental cats.

Materials and methods

In November 1985, large metacercariae were found in the crab *Sinopotamon chekiangenes*, in Ninghai County, Zhejiang, China. Seventy-nine metacercariae were isolated from 1,000 crabs. After measuring the metacercariae, 3 cats were infected with 20, 24, and 14 metacercariae, respectively. The infected cats were killed after 75, 90 and 110 days' infection. Adult worms

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paired and lived together in cysts in the lungs. Forty-four adult worms were recovered in all. Worm specimens were made for observation by fixing and pressing between two microscopic slides, dyeing with carmine, and counter-staining the tegumental spines with fast green. The primary identification was *Paragonimus harinasutai*. About 20 species of *Paragonimus* have been found in China, so great care should be taken with species identification. In 2004, a specimen was sent to Mahidol University, Thailand for re-identification, where it was confirmed as *Paragonimus harinasutai* Miyazaki & Vajrasthira, 1968. The specimen is kept in the Zhejiang Academy of Medical Sciences.

Results

Description

Adults are long-leafed in shape, sharp anteriorly and blunt posteriorly, measuring $11.46 \pm 0.94 \times 4.07 \pm 0.58$ mm (Fig 1A). The adult worm body proportion is 1 : 2.84 ± 0.23 . The oral sucker is $0.50 \pm 0.07 \times 0.67 \pm 0.09$ mm. The ventral sucker is a little larger than the oral, measuring $0.80 \pm 0.08 \times 0.82 \pm 0.09$ mm, located at the anterior middle third of the body. The ventral to oral sucker ratio is $1.26 \pm 0.12 : 1$. The pharynx is globular, measuring $0.31 \pm 0.06 \times$

0.42 ± 0.06 mm in length. The esophagus is 0.26 ± 0.10 mm. The ceca are bifurcated behind the esophagus, with 3-4 turns to the posterior end of the body. The excretory bladder extends to post bifurcation of the ceca.

The tegumental spines have a mixed-growth pattern: single-spaced before the acetabulum; mainly single, and some double, beside the acetabulum, typical double spines after the acetabulum and between the two testes. According to these 3 specimens, the tegumental-spine formula is 1, 1/2-4.

The ovary is on the posterior left of the ventral sucker, with 4-6 main lobes (Fig 1B). The lobes are subdivided into finger-like branches, forming into a coral-like shape, measuring $1.36 \pm 0.22 \times 1.30 \pm 0.22$ mm. The uterus is located opposite the ovary, rounding up to the acetabulum and open at the posterior border of the ventral sucker. The uterine mass measures 2.63 ± 0.61 mm. Yolk glands are very well developed, distributed profusely along both lateral sides of the body, from the anterior to the posterior ends. The testes are lumpish, with 4-5 branches; left testis $1.37 \pm 0.34 \times 0.87 \pm 0.18$ mm, right testis $1.23 \pm 0.20 \times 0.93 \pm 0.19$ mm (Fig 1C). The testes are located at the anterior of the posterior third of the body.

The eggs, which average $87.84 \pm 4.71 \times 58.00 \pm 3.36$ μ m, are golden brown in color. The widest is near the operculum. The eggshell is symmetrical and equal, thickened at the ab-opercular end. The egg is 26.91 ± 2.64 μ m in length. Fresh eggs include 8 cells.

The metacercaria is globular in shape and pinkish when fresh, with two cyst walls, measuring $646.09 \pm 90.53 \times 616.12 \pm 83.77$ μ m with the outer cyst wall, or $525.16 \pm 38.61 \times 500.19 \pm 31.71$ μ m without the outer cyst wall (Fig 2A, 2B). The inner cyst wall is 8.91 ± 3.51 μ m, and the outer cyst wall 21.17 ± 9.93 μ m in thickness. There is an interspace between the outer and inner cyst walls. The metacercaria folds and fills up the cyst. At a temperature of 35°C, metacercariae are very active and excyst readily.

Excysted metacercariae are pinkish in fresh specimens, measuring $0.88 \pm 0.22 \times 0.31 \pm 0.16$

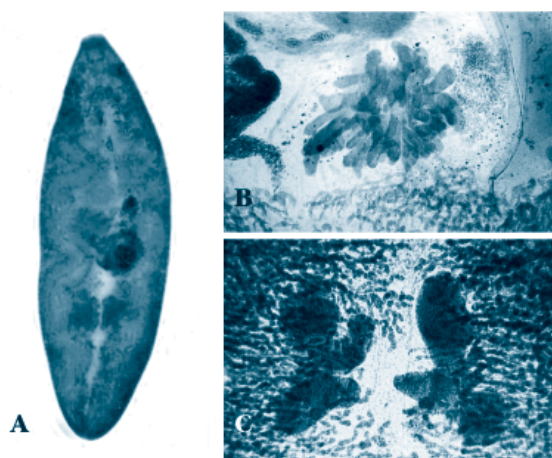


Fig 1 *Paragonimus harinasutai* adult: A. adult worm (whole mount); B. ovary; C. testes.

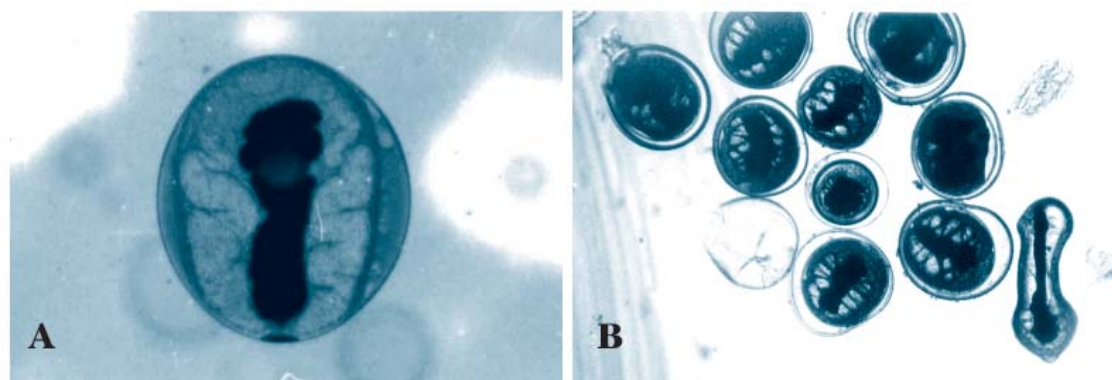


Fig 2 *Paragonimus harinasutai* metacercaria: **A.** encysted metacercaria without outer cyst wall; **B.** encysted and excysted metacercariae (small metacercaria in the middle is *P. westermani*).

Table 1 Comparison of the specimens from China and Thailand.

	Chinese specimen	Thai specimen
Final host	Cat (experimental)	Cat and dog (experimental)
Crab host	<i>Sinopotamon chekiangenes</i>	<i>Potamon smithianus</i>
Age of worm	75-110 days	381 days
Worm size (mm)	11.46 ± 0.94 x 4.07 ± 0.58	7.30-12.30 x 2.90-4.60
Length to width ratio	2.60-3.11 : 1	2.50-2.60 : 1
Oral sucker (L x W)	0.50 ± 0.07 x 0.67 ± 0.09	0.37-0.46 x 0.49-0.61 (average 0.48 x 0.58)
Ventral sucker (L x W)	0.80 ± 0.08 x 0.82 ± 0.09	0.53-0.63 x 0.53-0.71 (average 0.60 x 0.66)
Location of ventral sucker	34.60%	anterior
Ratio of ventral to oral sucker	1.04-1.30 : 1	1.08-1.16 : 1
Ovary	4-6 lobes with subdivided branches 1.35 ± 0.22 x 1.30 ± 0.22	Moderate branching 0.63-1.19 x 0.60-0.73
Testes	4-5 lumpish branches L, 1.37 ± 0.34 x 0.87 ± 0.18 R, 1.23 ± 0.20 x 0.93 ± 0.19	Simple branching L, 0.94-1.22 x 0.58-0.73 R, 1.05-1.39 x 0.68
Metacercaria (µm)	Pinkish with 2 cyst walls 646.09 ± 90.53 x 616.12 ± 83.77 (with outer cyst wall) 525.16 ± 38.61 x 500.19 ± 31.71 (without outer cyst wall)	Pinkish with 2 cyst walls 533-666 x 513-649 (without outer cyst wall) Average 601 x 579
Egg	78.58-96.44 x 53.58-65.19	79.40-99.80 x 46.10-56.30
Tegumental spines	Mixed	Single-spaced

mm. The acetabulum is $0.11 \pm 0.01 \times 0.01 \pm 0.01$ mm. The stylet is about 0.08 mm. The ceca are wide along the side of the body to the posterior end. The bladder is thin and reaches the bifurcation of the ceca. A flame cell is not observable.

A comparison of the specimens from China and Thailand is shown in Table 1.

Discussion

Most features of the Chinese and Thai specimens are similar. However, the Thai specimens are smaller than the Chinese, which may be due to the pressure excited during fixing. The specimens from the two countries differ in tegumental spine arrangement, described as single-spaced spines in the Thai specimen and mixed single and double-spaced spines in the Chinese specimens. Actually, in the Thai specimen, there are double-spaced spines around the ventral sucker areas, but in other parts of the body they are single-spaced.

Comparison with other species, *P. yunnanensis* Ho *et al*, 1959 and *P. menglaensis* Chung *et al*, 1964, have been described as large metacercariae. The metacercaria of *P. menglaensis* is larger than that of *P. yunnanensis*, and *P. yunnanensis* is almost as large as *P. harinasutai*. However, the cyst walls of *P. menglaensis* and *P. yunnanensis* are thinner. Moreover, the form of *P. yunnanensis* metacercariae changes by the movement of larvae inside, and the arrangement of the tegumental spines in the adults are different. In China, *P. skrjabini* resembles *P. harinasutai*, except for body size; *P. skrjabini* is more slender than *P. harinasutai* and the metacercariae of the two species are easily differentiated [5].

The crab, *Sinopotamon chekiangenes*, acts as an intermediate host harboring *P. harinasutai* metacercariae in Ninghai County, Zhejiang Province, China. In Thailand, two species of crabs, *Larnaudia beusekoma* (*Tiwaripotamon beusekoma*) and *Siamthelphusa paviei*, collected from the running outlets of a waterfall in Nakhon Nayok Province, harbored *P. harinasutai* metacercariae. Cats and dogs served as definitive

hosts in experimental studies [6]. However, a dog host has not been used experimentally in China. Although this species of *Paragonimus* has presented in Thailand, paragonimiasis *harinasutai* has never been reported in humans. Due to the few variations in morphology found between the specimens from China and Thailand, the specimens from China have been identified as *P. harinasutai*. Further studies on the phylogenomic positions of the specimens from both countries are needed to reconfirm their identification.

Acknowledgements

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